

In the Claims:

This listing of claims will replace all prior versions and listings of claims in this application.

1-48 (canceled).

49 (new). A kit useful for directed cloning or subcloning of a target DNA molecule comprising in one or more containers:

- a) a double-stranded DNA vector useful for directed cloning and subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; and
- b) a first double-stranded adaptor oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first sequence and a second sequence, wherein said first nucleotide sequence is homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence is homologous to the nucleotide sequence of a first terminus on a target DNA strand; and
- c) a second double-stranded adaptor oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5': a third nucleotide sequence and a fourth nucleotide sequence, wherein said third nucleotide sequence is homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence is homologous to the nucleotide sequence of a second terminus on said target DNA strand.

50 (new). The kit of claim 49 which further comprises

- d) a cell containing a bacterial recombinase.

- 51 (new). The kit of claim 50 wherein the cell is an *E. coli* cell.
- 52 (new). The kit of claim 51 wherein the cell is a frozen cell competent for uptake of DNA.
- 53 (new). The kit of claim 49 wherein the DNA vector is purified.
- 54 (new). The kit of claim 49 wherein the DNA vector, double-stranded oligonucleotide, and the second double-stranded nucleotide are purified.
- 55 (new). The kit of claim 49 wherein the target DNA molecule comprises bacterial,
- 56 (new). The kit of claim 49 wherein the target DNA molecule comprises a genetic mutation or polymorphism known or suspected to be associated with a disorder or disease.
- 57 (new). The kit of claim 49 wherein the bacterial recombinase is RecE/T or Red α / β recombinase or both RecE/T and Red α / β recombinases.
58. (new). The kit of claim 49 wherein the first and second double-stranded oligonucleotide have nucleotide sequence homology to a BAC, PAC, lambda, plasmid or YAC based cloning vector.
- 59 (new). The kit of any one of claims 49 to 58 in which said vector is a linear vector.
- 60 (new). A method for making a double-stranded DNA vector useful for directed cloning or subcloning of a target DNA molecule of interest, comprising incorporating first and second homology arms into a double-stranded DNA molecule, wherein the double-stranded DNA molecule comprises an origin of replication, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication, and a second homology arm, such that the nucleotide sequence of the first homology arm on a first vector DNA strand is

homologous to the sequence of the first terminus on a first target DNA strand, and the nucleotide sequence of the second homology arm on the first vector DNA strand is homologous to the nucleotide sequence of the second terminus on the first target DNA strand, wherein said target DNA molecule comprises a target DNA sequence and two termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, the target DNA sequence, and a second terminus.

61 (new). A method for making a double-stranded DNA vector useful for directed cloning or subcloning of a target DNA molecule of interest, comprising:

- a) choosing two homology arms, such that the sequence of the first homology arm is designed to be homologous to the sequence of a first terminus on a target DNA strand, and the sequence of the second homology arm is designed to be homologous to the sequence of second terminus on the target DNA strand, wherein the target DNA comprises a target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along a target DNA strand: the first terminus, the target DNA sequence, and the second terminus, the orientation of the two arms relative to the desired insert being the same as the orientation of the homologous sequences relative to the target DNA so that recombination between the homology arms and the first and second termini results in the desired target sequence being inserted between the homology arms; and
- b) constructing a vector by incorporating the two homology arms into a DNA molecule comprising an origin of replication in the following order from 5' to 3' long a vector DNA strand: the first homology arm, the origin of replication and the second homology arm.

62 (new). The method of claim 60 or 61 wherein the origin of replication is a bacterial origin of replication.

63 (new). The method of claim 60 or 61 wherein the origin of replication functions in *E. coli*.

64 (new). The method of claim 60 or 61 wherein the origin of replication functions in a mammalian cell.

65 (new). A method for making a recombinant DNA molecule comprising making a double-stranded vector according to the method of claim 60 or 61, further comprising the steps of:

- a) introducing the target DNA molecule into a cell, said cell containing the vector and expressing a bacterial recombinase; and
- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

66 (new). A method for making a recombinant DNA molecule comprising making a double-stranded vector according to the method of claim 60 or 61, further comprising the steps of:

- a) introducing the target DNA molecule and the vector into a cell, said cell expressing a bacterial recombinase; and
- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

67 (new). A method for making a recombinant DNA molecule comprising:

- a) preparing first and second double-stranded oligonucleotides, wherein said first oligonucleotide comprises a first oligonucleotide DNA strand comprising, from 3' to 5', a first nucleotide sequence and a second nucleotide sequence, said first nucleotide sequence being homologous to the nucleotide sequence of a first homology arm on a vector DNA strand and said second nucleotide sequence being homologous to the nucleotide sequence of a first terminus on a target DNA strand, and wherein said second oligonucleotide comprises a second oligonucleotide strand comprising, from 3' to 5', a third nucleotide sequence and

a fourth nucleotide sequence, said third nucleotide sequence being homologous to the nucleotide sequence of a second homology arm on said vector DNA strand and said fourth nucleotide sequence being homologous to the nucleotide sequence of a second terminus on said target DNA strand; wherein said vector comprises an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, an origin of replication and a second homology arm, wherein said target DNA comprises a target DNA sequence and two termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, a target DNA sequence and a second terminus, the orientation of the two arms relative to the desired insert being the same as the orientation of the homologous sequences relative to the target DNA so that recombination between the homology arms and the first and second termini results in the desired target sequence being inserted between the homology arms;

- b) introducing the target DNA and the first and second double-stranded oligonucleotide into a cell, said cell containing a vector and expressing a bacterial recombinase; and
- c) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

68 (new). A method for making a recombinant DNA molecule comprising:

- a) preparing first and second double-stranded oligonucleotides, wherein said first oligonucleotide comprises a first oligonucleotide DNA strand comprising, from 3' to 5', a first nucleotide sequence and a second nucleotide sequence, said first nucleotide sequence being homologous to the nucleotide sequence of a first homology arm on a vector DNA strand and said second nucleotide sequence being homologous to the nucleotide sequence of a first terminus on a target DNA strand, and wherein said second oligonucleotide comprises a second oligonucleotide strand comprising, from 3' to 5', a third nucleotide sequence and fourth nucleotide sequence, said third nucleotide sequence being homologous to the nucleotide sequence of a second homology arm on said vector DNA strand and said fourth nucleotide sequence being homologous to the nucleotide sequence

of a second terminus on said target DNA strand, the orientation of the two arms relative to the desired insert being the same as the orientation of the homologous sequences relative to the target DNA so that recombination between the homology arms and the first and second termini results in the desired target sequence being inserted between the homology arms;

- b) introducing the vector, the target DNA, and the first and second double-stranded oligonucleotide into a cell expressing a bacterial recombinase; and
- c) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

69 (new). A method for making a recombinant DNA molecule comprising culturing a bacterial cell that expresses a bacterial recombinase or functional equivalent thereof, said bacterial cell containing

- a) the target DNA comprising a first double-stranded terminus and a second double-stranded terminus,
- b) a vector DNA comprising, in the following order along the vector DNA strand:
 - (i) a first double-stranded homology arm;
 - (ii) an origin of replication; and
 - (iii) a second double-stranded homology arm,such that the sequence of a vector DNA strand of the first homology arm is designed to be homologous to the sequence of a target DNA strand of the first terminus, and the sequence of a vector DNA strand of the second homology arm is designed to be homologous to the sequence of the target DNA strand of the second terminus, such that the target DNA is inserted into the vector DNA between the homology arms; the orientation of the two arms relative to the desired insert being the same as the orientation of the homologous sequences relative to the target DNA so that recombination between the homology arms and the first and second termini results in the desired target sequence being inserted between the homology arms; and
- c) isolating from the cell a recombinant DNA molecule that comprises the target DNA sequence inserted into the vector.

70 (new). A method for making a recombinant DNA molecule comprising:

- a) introducing a first and second strand of a double-stranded target DNA molecule into a cell expressing a bacterial recombinase, said target DNA comprising a target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along said first target DNA strand: a first terminus, a target DNA sequence, and a second terminus; said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; such that the sequence of the first homology arm on said vector DNA strand is designed to be homologous to the sequence of the first terminus on said first target DNA strand, and the sequence of the second homology arm on said vector DNA strand is designed to be homologous to the sequence of the second terminus on said target DNA strand; the orientation of the two arms relative to the desired insert being the same as the orientation of the homologous sequences relative to the target DNA so that recombination between the homology arms and the first and second termini results in the desired target sequence being inserted between the homology arms;
- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur; and
- c) isolating from the cell a recombinant DNA molecule that comprises the target DNA sequence inserted into the vector.

71 (new). A method for making a recombinant DNA molecule comprising:

- a) introducing a first and a second strand of a double-stranded vector and first and a second strand of a double-stranded target DNA into a cell expressing a bacterial recombinase, said double-stranded vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a first vector DNA strand: a first homology arm, the origin of replication and second homology arm, said double-stranded target DNA comprising a target DNA sequence and two termini, in the following order, from 3' to 5' along said first target DNA strand: a

first terminus, a target DNA sequence; and a second terminus; such that the nucleotide sequence of the first homology arm on said vector DNA strand is designed to be homologous to the nucleotide sequence of the first terminus on said target DNA strand, and the nucleotide sequence of the second homology arm on said vector DNA strand is designed to be homologous to the sequence of the second terminus on said target DNA strand; the orientation of the two arms relative to the desired insert being the same as the orientation of the homologous sequences relative to the target DNA so that recombination between the homology arms and the first and second termini results in the desired target sequence being inserted between the homology arms;

- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur; and
- c) isolating from the cell a recombinant DNA molecule that comprises the target DNA sequence inserted into the vector.

72 (new). The method according to any one of claims 60, 61, and 67-71, wherein the vector DNA does not contain a directly repeated sequence of five or more bases between (i) the homology arm sequences and the sequences that encode the origin of replication; (ii) the homology arm sequences and the selectable marker or (iii) the homology arm sequences and the ends of a linear DNA vector.

73 (new). A method according to any of claims 60, 61, 67, 68, 69, 70, and 71, said method comprising using a kit useful for directed cloning or subcloning of a target DNA molecule, said kit comprising in one or more containers:

- a) a double-stranded DNA vector useful for directed cloning and subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; and
- b) a first double-stranded adaptor oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first sequence

and a second sequence, wherein said first nucleotide sequence is homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence is homologous to the nucleotide sequence of a first terminus on a target DNA strand; and

- c) a second double-stranded adaptor oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5': a third nucleotide sequence and a fourth nucleotide sequence, wherein said third nucleotide sequence is homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence is homologous to the nucleotide sequence of a second terminus on said target DNA strand.

74 (new). The method of claim 73 wherein said kit further comprises
d) a cell containing a bacterial recombinase.

75 (new). The method of claim 74 wherein the cell is an *E. coli* cell.

76 (new). The method of claim 75 wherein the cell is a frozen cell competent for uptake of DNA.

77 (new). The method of claim 73 wherein the DNA vector is purified.

78 (new). The method of claim 73 wherein the DNA vector, double-stranded oligonucleotide, and the second double-stranded nucleotide are purified.

79 (new). The method of claim 73 wherein the target DNA molecule comprises bacterial, viral, parasite, or protozoan DNA.

80 (new). The method of claim 73 wherein the target DNA molecule comprises a genetic mutation or polymorphism known or suspected to be associated with a disorder or disease.

81 (new). The method of claim 73 wherein the bacterial recombinase is RecE/T or Red α / β recombinase or both RecE/T and Red α / β recombinases.

82. (new). The method of claim 73 wherein the first and second double-stranded oligonucleotide have nucleotide sequence homology to a BAC, PAC, lambda, plasmid or YAC based cloning vector.

83 (new). The method of any one of claims 73 to 82 in which said vector is a linear vector.